LJI BSL-2 LEVEL CELL SORTING INFO & PROCEDURES

High-speed cell sorters use higher system pressures and higher drop drive frequencies, which produce smaller droplets and satellite drops. During instrument failure, e.g. partial blockage of the nozzle, the generation of secondary aerosols can occur (1). The potential exposure to escaped aerosols may be a health risk to sort operators. Aerosol containment must be properly assessed.

Sorting of live, unfixed cells that contain a potential pathogen need to be treated at the appropriate biosafety level. A thorough risk assessment and extra safety precautions must be taken for cell sorting with unfixed samples designated at the BSL-2 level, or samples exposed to or infected with pathogens designated at the BSL-2 level.

The following can be used as a guideline for initial biosafety level designation:

BSL-1: Cells from murine or other non-human/non-primate species that HAVE NOT been exposed to any microbial agent (e.g. viral, bacterial, fungal, protozoan, parasitic) and HAVE NOT been genetically modified, cells from murine or other non-human primate species that HAVE BEEN genetically modified using non-viral methods

	Exempt BSL-1	**BSL-1**	**BSL-2**
Cell Types	Wild type (WT) cells from murine or other non-human/non-primate species that have **NOT** been exposed to any microbial agent (e.g. viral, bacterial, fungal, protozoan, parasitic) **AND** have **NOT** been genetically modified. **OR** Cells determined by UW EH&S and the UW IBC to be recombinant NIH-exempt BSL-1.	Cells from murine or other non-human/non-primate species that have **NOT** been exposed to any microbial agent (e.g. viral, bacterial, fungal, protozoan, parasitic) but **HAVE BEEN** genetically modified using non-viral methods (i.e. cells from transgenic animals or cells treated with nucleic acids) **OR** .	Cells from human or non-human primates **OR** Cells that have been genetically modified using viral methods **OR** Cells exposed to microbial agents (e.g. viral, bacterial, fungal, protozoan, parasitic) **AND** Have been approved by UW EH&S and UW IBC for BSL-2 containment and sorting

The FACSAria Fusion is completely contained in a biosafety hood for sorts requiring BSL-2 with BSL-3 safety precautions. The FACSAria-1 and FACSAria-3 are equipped with the Aerosol Management System, which evacuates the sort collection chamber and traps aerosols during sorting. The latest generation of FACSAria cell sorters feature a much improved fluidics design and added safety elements for BSL-2 level sorting. The fluidics pathway from sample injection

chamber to the sort collection tubes is completely enclosed. The simplified fluidics path allows for easier decontamination after infectious sorting.

For BSL-2 level sorting, please follow the procedures below:

Biohazard Form & Scheduling

All analyzer and sorter scheduling is completed through iLab. Please complete all the required fields in iLab for Biohazard information for each reservation. Provide as much information as possible with your sort request including: cell type, pathogen, days post infection, total cell #, total sort time needed, and fluorochrome combination with your sort request.
* **Please include one hour of decontamination time to your reservation.**

Sample Handling and Containment

1. All samples with cells containing potentially infectious agents must be in tubes with snap caps or lids closed tightly to avoid spills.

2. The tubes must also be enclosed in secondary containment when transporting the samples to the sorting lab. The secondary containment (pink cooler) is provided to each laboratory labeled with Biohazard and the infectious agent.

3. Any leftover sample tubes must be disposed of in the proper biohazard waste container.

Personal Protective Equipment (PPE)

Please follow EH&S procedures for working with BSL-2 classified agents.

Sort operator and users must use a laboratory coat and disposable gloves at all times when handling samples and when operating the cell sorter. With any infectious agent requiring BSL-3 practices, face protection is **REQUIRED.**

Sorter Decontamination

The decontamination cycle is performed by Flow Cytometry Staff, and replaces the fluidics path with 70% EtOH . After this cycle, the fluidics path uses sterile sheath to rinse out the 70% EtOH. The decontamination cycle takes about 1 hour.